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Long term geological record of a global deep subsurface microbial habitat in sand injection complexes

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There is extensive evidence from drilling into continental margins for microbial colonization of a deep biosphere. However it is difficult to prove deep biosphere activity in the geological record, where evidence for life is dominated by the remains of organic matter buried after deposition at the surface. Nevertheless we propose that natural injections of sand into muddy strata at continental margins represent an excellent habitat opportunity for deep microbial activity down to several kilometres' present day depth. Sulphur isotope data for iron sulphides precipitated soon after injection indicate consistent microbial sulphate reduction through the geological record. The complexes are favourable sites for colonization, because high permeability and extensive sand/mud interface allow ready availability of electron donors and nutrients. The measured examples of iron sulphide in injected sands extend back to the Proterozoic, and show that injected sand complexes have been a long-term environment for deep subsurface microbial colonization.

The injection of sand into mudrocks (Fig. 1) occurs in sedimentary basins worldwide and throughout the geological record[1,2]. The occurrence of sand injections dates back to the Archean[3]. Despite an extensive literature, their global importance has only recently come into focus. Driven by abundant evidence made available from hydrocarbon exploration[1], and to a lesser extent from mineral exploration[4], it is now evident that sand injection complexes are found in sedimentary basins at every continental margin[2]. As these sediment packages are typically mixtures of sand and mud, there is a natural susceptibility to injection because of the different permeabilities to fluid flow (see below). Injection occurs on a scale of up to hundreds of millions of cubic metres, down to several kilometres' depth, and is a major mechanism for cross-formational sediment and fluid movement in the crust. Several review volumes have documented the widespread occurrence of these complexes[1,2,5]. Where they are well-studied, there is also evidence for multiple episodes of injection within the history of a single region[2]. The scale of the phenomenon is so great that injected sands are now targets for the drilling and extraction of huge oil reserves[6,7]. The largest injection structures appear in seismic profiles, and in the North Sea may contain millions of barrels of oil in a single structure[1] and billions of barrels in total[2,6]. The scale of occurrence indicates a significant global cause of cross-formational flow of fluid and sediment.

The recognition of this globally distributed subsurface network of sand bodies, known as sand injectites[1], suggests an important, but hitherto little recognized, residence for a deep biosphere. The deep biosphere, encompassing life beneath a depth of 1 m below the surface[8] accounts for a significant proportion of life on Earth, estimated at one tenth to one half of the total[9–13]. Investigations of the deep biosphere generally extend down several hundred metres below the ocean floor and at continental margins, while it has been proved to several kilometres depth below the continental surface[14–16]. Evidence for a deep biosphere in the geological record is potentially available from the occurrence of microfossils in fracture systems, and biological microfabrics and isotopic fractionation in mineral precipitates in subsurface settings[17–20]. This study uses the isotopic composition of sulphur in pyrite within ancient injected sands to demonstrate a geological record of a subsurface microbial habitat.

Investigations of subsurface life show that its distribution is controlled by the availability of electron donors and acceptors, and porosity and also enhanced by flow of fluid to maintain nutrient supply[21–24]. These factors are interrelated, and combine optimally at mudrock-sandstone interfaces, where electron donors diffuse from the mudrock into relatively porous sandstone[21–25]. The cross-cutting nature of sand injections through mudrocks provides extensive mudrock-sandstone interfaces, suggesting that they would provide sites for colonization by

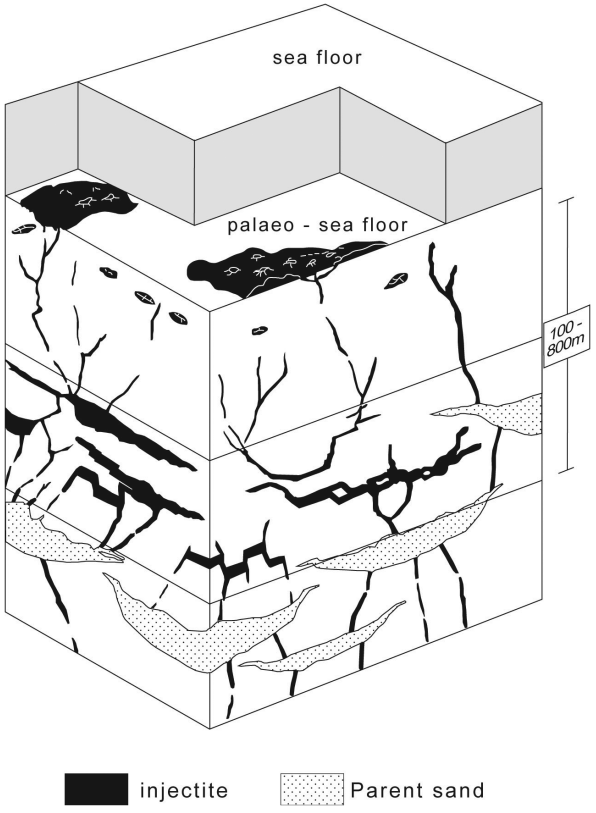

Figure 1 | Schematic section through injectite complex showing extensive sand-mud interface. Host to parent and injectite sand (unornamented) is mud. (adapted from ref. 7).

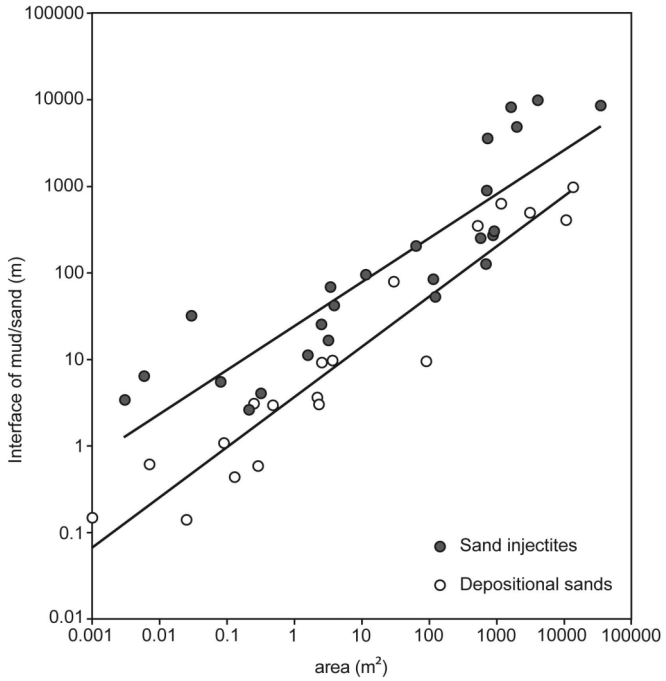

Figure 2 | Sand/mud interface length relative to area, for sand injectites and depositional sands. Data for interface length and area from published images, and converted to line drawings for image analysis (details in Supplementary Information). 2-D data represent interface area for sediment volume in 3-D, and indicate an order of magnitude more interface for injectites compared to depositional sands.

subsurface microbial life. Evidence for the widespread colonization of this potential habitat was sought in the distribution of bacteriogenic sulphide precipitation.

A commonly encountered characteristic of injected sands in the geological record is their partial cementation by the iron sulphide mineral pyrite (Twenty examples listed in Supplementary Information). The pyrite commonly occurs at, and adjacent to, the interface between sandstone and mudrock. This is consistent with a prediction that bacteriogenic pyrite should occur in sandstone adjacent to sandstone-mudrock interfaces[25], where the porosity can accommodate microbes, and nutrients diffuse from the mudrock[22–25]. In support of this model, two to three orders of magnitude greater sulphate reduction have been recorded at such interfaces[22]. Bacteriogenic pyrite can be distinguished by its sulphur isotope composition (Methods). Five samples of pyrite from each of four injectite systems, representing different ages from Proterozoic to Oligocene and a diversity of environments (locality details in Supplementary Information), were analysed and compared to the compositions of contemporaneous seawater sulphate. The fractionation between sulphate and sulphide was used as a measure of whether microbial sulphate reduction was responsible for pyrite precipitation. The sampled injectites were:

(i) Lower Oligocene (30 Ma) injectites in a glacio-marine succession, Ross Sea, Antarctica[26]. Sandstone dykes were sampled from depth interval 447.06 to 447.11 m, core CRP-2/2A, Victoria Land. The dykes are centimeter-scale width, contain pyrite cement at this and several other levels, and have irregular margins with the mudrock host[26].

(ii) Carboniferous (326 Ma) injectites in a deltaic succession in County Leitrim, Republic of Ireland[27]. Sandstone dykes up to 1.2 m width were sampled at Lugasnaghta, County Leitrim, where they are dolomitic, with pyrite concentrated at the margins with grey-black shale[27]. A coeval succession (Pendleian stage), 400 km distant in England, also contains pyrite-bearing injectites[28].

(iii) Lower Ordovician (466 Ma) injectites in deep marine turbidite/shale sequence, Counties Mayo and Galway, Republic of Ireland[29,30]. Sandstone dykes and sills, mostly of centimetre-scale width, occur with irregular margins in black shales at several levels of the Ordovician sequence, and contain patchy pyrite cement where sampled at Doo Lough, County Mayo.

(iv) Proterozoic (~615 Ma) injectites in marine shelf sequence, Dalradian Supergroup, Argyll, UK[31]. Sandstone dykes were sampled at the Point of Knap, Argyll, where they are up to 0.5 m wide, cutting carbonaceous mudrocks, with pyrite close to the margins.

These case studies represent large-scale long-lived injection systems at continental margins. The Proterozoic and Ordovician injectites occur at intervals through several kilometres of stratigraphy in both cases, and the Oligocene injectite-bearing section is at least 400 m thick. The two coeval Carboniferous examples formed 400 km apart in a huge deltaic complex.

Results

Enhanced mudrock-sandstone interface. The enhanced interface made available by the sand injections is evident in a new compilation of data for interface area per volume of sediment (Fig. 2). Over a wide range of scales, the sand injections exhibit an order of magnitude more mudrock-sandstone interface than non-injected sand-mud sequences (measurement against volume takes account of scale-dependent resolution of images). In addition to mudrock-sandstone margins, there is much interface between entrained mudstone clasts and sand[32]. The injection process causes mud-grade wallrock to be incorporated as small clasts within the sand[32].

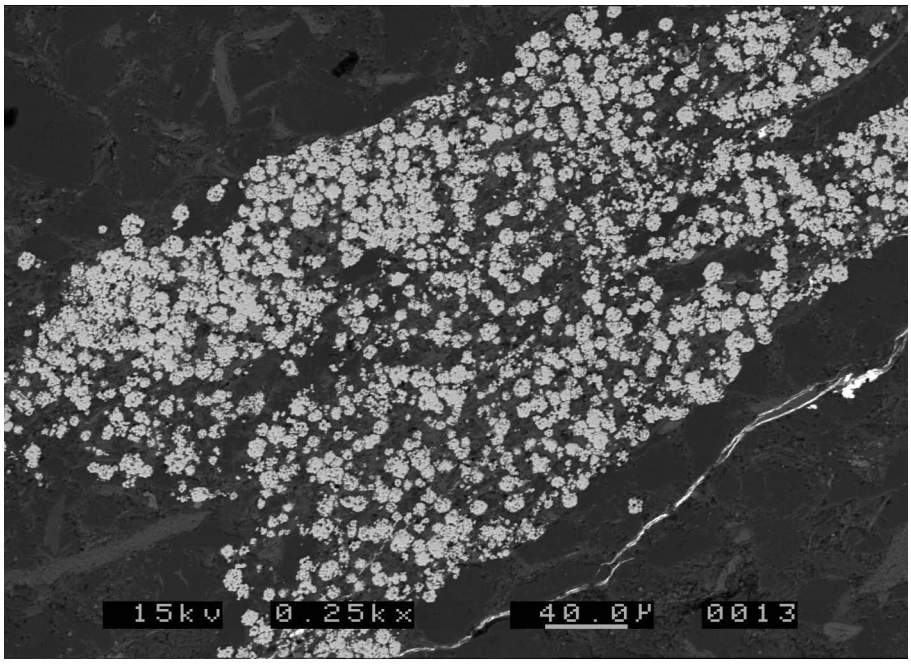

Figure 3 | Occurrence of framboidal pyrite from sandstone-mudrock interface in injectite complex. Scanning electron micrograph, Ordovician, Co. Galway, Ireland.

Pyrite occurrence. The pyrite was precipitated very early, determined by microscopy studies of mineral paragenesis, and an open (pre-compaction) fabric at the time of precipitation, before other phases precipitated by continuing fluid flow through the injectites[33]. Also, in the case of the youngest example the pyrite must have precipitated within the 30 million years age of the host rock. The pyrite consists of fine (micro-scale) crystals which, where densely distributed, have merged into larger masses. Where the pyrite occurs on the mudrock side of the interface, it exhibits a framboidal texture (Fig. 3), which is typical, although not diagnostic in itself, of precipitates from microbial sulphate reduction[34]. Our experience of examining many thousands of metres of cored sandstones in oilfields suggests that sulphides are several times more likely to be encountered in injected sands compared with depositional sands.

Isotopic composition of pyrite in injectites. In each of the four case studies, the injection-related pyrite sulphur was found to have an isotopically light composition (Fig. 4; Table 1). The mean values and standard deviations of the sets of five measurements are -14.3 ± 2.0‰ (Oligocene), -11.8 ± 1.0‰ (Carboniferous), -2.5 ± 0.8‰ (Ordovician) and -12.0 ± 1.3‰ (Proterozoic). The isotopic fractionation between contemporary seawater sulphate and associated pyrite, calculated using the mean $\delta^{34}S$ values of pyrite and the established seawater composition curve[35] are approximately 35‰ (Oligocene), 25‰ (Carboniferous), 30‰ (Ordovician) and 40‰ (Proterozoic). In each case these fractionation values clearly exceed the maximum known kinetic isotope fractionation of ~20‰ that is possible from non-biological mechanisms, such as abiological thermochemical sulphate reduction[36]. The precision of measurement is ±0.2 per mil, and the uncertainty in seawater composition is within 1‰ for the Oligocene and Carboniferous, and within 3‰ for the Ordovician and Proterozoic[35]: These values do not compromise the magnitude of the fractionations. The occurrence of the pyrite early in the paragenetic sequences implies that the compositions of parent seawater were close to those at the time of deposition. However, in each case precipitation at any stage during the subsequent 30 million years would involve seawater of similar composition (Fig. 4), so the precision of timing of pyrite precipitation is not a major constraint. Furthermore three of the four data sets are more than 20‰ lighter than the lightest composition of seawater at any stage during the last 800 million years (Fig. 4). The data thus strongly indicate a long-term geological record of microbial habitat in the deep subsurface associated with injectites. The Proterozoic occurrence is in every way comparable to the Phanerozoic occurrences. In addition, one of the largest base metal (zinc-lead) deposits in the world, in Yunnan Province, China, in which sand injection through other sedimentary rocks was fundamentally important to ore fluid circulation[4], yields mean sulphide compositions[37] over 30 per mil lighter than contemporary Paleocene seawater, providing further evidence for a habitat with a long geological record, although in this case, the mobilisation of evaporite sulphate within the basin may have added to account for a large tonnage of bacteriogenic sulphide. The demonstration of a microbial signature in each of the case studies suggests that the common occurrence of pyrite in other injectites (see Supplementary Information) similarly reflects microbial activity.

Discussion

The mudrock-sandstone interface in injected sands is extensive. As the mud-sand interface is shown in modern subsurface environments to be a critical control on the location of microbes[21], the greatly enhanced area presented by injectite networks thus offers extensive opportunities for microbial colonization. Experimental addition of ground mudrock into sand promoted microbial growth in the sand[22], suggesting that the comparable natural admixture in injected sands may also stimulate microbial activity.

The petrophysical characteristics of sand injectite networks enhance the potential of the injectites as a habitat for microbial activity. Injected sandstones retain porosities of over 30% (refs. 2,7,32), and therefore allow cross-formational fluid flow through the mudrocks they penetrate. This results in 5 to 6 orders of magnitude increase in vertical permeability, so has a huge influence on rates of fluid movement[7]. The increase in flow also allows enhanced drainage of fluids from mudrocks into sandstones, including the organic acids and other compounds that fuel microbial activity. In each of the studied cases, the mudrocks hosting the injectites contained marine organic matter that could provide nutrients and

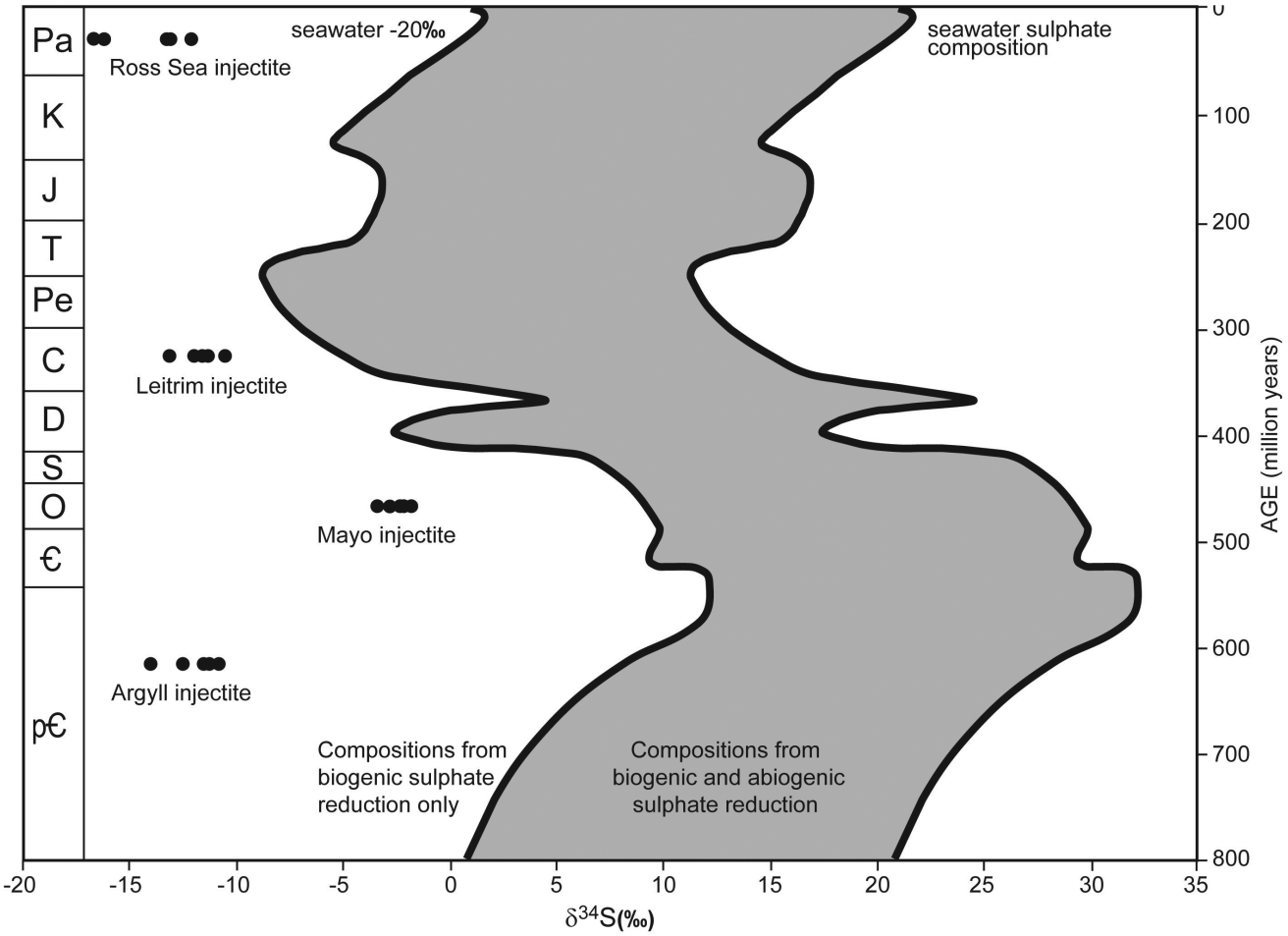

Figure 4 | Isotopic composition of pyrite sulphur in four injectites (five samples each). Data are compared with seawater sulphate over last 800 million years, and in each case is more than 20‰ lighter than contemporary seawater, indicating microbially-induced isotopic fractionation. Shaded region indicates compositional field which could be explained by both biogenic and abiogenic sulphate reduction. Seawater composition from ref. 35 (modified for timescale of ref. 52).

carbon to a microbial community in the injectite. Cores through younger successions show the importance of mudrock organic matter in the location of microbial activity[8,38]. Data from oilfields show that injected sands remain porous and permeable for tens of millions of years after injection[2]. For example, many injectites in the North Sea of Paleocene age still have potential as oil reservoirs. Throughout their histories of progressive burial and subsequent uplift, these sand injectites are likely to remain within a depth range of up to 4 km, over which continuing compaction of mudrock lithologies would continue to release organic acids and inorganic nutrients[21,24]. This would allow progressive increase in biomass, rather than the biomass stagnation inevitable in an isolated subsurface environment. Although most injection takes place within a kilometre of the surface[6,33], where over 95% of subsurface prokaryotes probably reside[9], the injectites represent long-term habitats with available space and nutrition.

Continental margins are the locus of most of the world's sedimentation[39]. The widespread occurrence of mud-sand mixtures in this setting results in the common occurrence of injectites in sedimentary basins. The discovery of microbial populations in deeply buried sandstones[22] shows that normally deposited sandstone beds may be a deep biosphere habitat. However, the greater interface with mudrock, and greater rates of fluid movement, in injected sands make them a much more favourable habitat. This is supported by our observation that sulphide cementation is much more prevalent in injected sands compared with depositional sands. Similarly, in some ancient sequences, pyrite is present in the injectites, but not the depositional sands[28], consistent with an advantageous habitat in injectites. This contrast also strongly suggests that the sulphide was formed in the injected sands, not formed elsewhere then transported, otherwise we would expect a more uniform precipitation of pyrite. The precipitation of pyrite in sandstone requires an adequate supply of seawater sulphate, which is the predominant source of sulphur in sedimentary pyrite[34]. The sulphate available to the bacteria was not a residue of sulphate spent from earlier reduction in the shallow sedimentary environment, as this sulphate would likely be diminished in concentration, and isotopically heavy as a result of closed system bacteriogenic reduction[40]. Instead, there was a readily available source of sulphate retaining the original isotopic composition of seawater sulphate, reflected in the open system S isotope fractionations measured in the pyrites. Sulphide with a light composition could also be formed at methane-sulphate transition zones where the sulphate concentration is low, but the abundant pyrite in the injectites shows that the systems were not sulphate-limited, so this is not a viable alternative in these cases. In normal marine sediment, pyrite formed in the transition zone tends to be heavy[41], unlike our values. Also, in the specific case of the Irish Carboniferous example, we know from the many sulphur isotope studies of Irish ore mineralization that the fluids circulating in the basin were dominated by evaporated Carboniferous seawater, and the average fractionation between contemporaneous seawater sulphate and ore sulphide (representing many million tonnes of bacteriogenic sulphide in the basin, at ore grade alone) is 36‰ (ref. 42). This extent of fractionation

Table 1 | Sulfur isotope data for pyrite in sand injectites

Locality	Setting	Mudrock TOC Range (%)	Pyrite residence	Data $\delta^{34}S$ (‰)	Technique	Contemporary seawater composition approx. $\delta^{34}S$ (‰)
Oligocene, Ross Sea, Antarctica	Glaciomarine sandstone & mudrock	<0.7	Cement in sandstone dykes	−13.2 −16.2 −16.6 −12.2 −13.1	conventional	+20
Carboniferous, County Leitrim, Ireland	Deltaic sandstone & marine mudrock	0.5–2.0	Cement at sandstone dyke/mudrock margins	−13.4 −10.6 −11.6 −12.2 −11.4	conventional	+15
Ordovician, Co. Mayo, Ireland	Turbidite sandstone & deep marine mudrock	0.5–1.0	Cement in sandstone dykes	−1.7 −3.9 −2.7 −2.1 −2.3	laser	+29
Neoproterozoic Argyll, Scotland	Shelf sandstone and marine mudrock	1.0–5.0	Concretions at margins of sandstone dykes	−12.5 −11.3 −11.5 −14.0 −10.8	laser	+28

is what we see in the Leitrim injectites, and supports our argument for an origin through microbial sulphate reduction.

Many of the North Sea injected sandstones exhibit partial cementation by pyrite[33,43], up to 10% volume at the cubic decimeter scale. A volume of pyrite requires a minimum of almost 3000 volumes of seawater to supply the sulphur (see Supporting Information), and this is a conservative value based on the current composition of seawater. Sand injection complexes are much more likely than depositional sands to experience the fluid flow required to leave a record of microbial sulphate reduction in the form of pyrite. While the sulphide was available in the sandstone pore water, the iron was probably supplied from the compacting mudrocks. The sandstone mineralogy is almost exclusively quartz, but mudrocks contain iron-rich clay minerals that can release iron during clay diagenesis. For example the host mudrocks in the Oligocene Ross Sea succession contain iron-rich smectites[44].

To some extent, sand injection is a normal consequence of alternating sand (high-permeability) and mud (low-permeability) sedimentary successions. Build-up of pore-fluid pressure during burial causes hydraulic fracturing in the mudrock when it exceeds the fracture pressure, and the pore pressure can then cause sand to flow into the fracture system[7]. In some cases, sand injection may be triggered by seismic activity, although the evidence for this is limited[2,7]. Fracture systems in general enhance subsurface habitable environments, as they confer space, generate water-rock reaction surfaces, and allow circulation of fluid and entrained nutrients[17,20,45]. The evidence for microbial sulphate reduction associated with injectites suggests that they are part of a family of fracture-related settings, which might also include fault zones, impact-induced fractures and hydrothermal veins, which together form a wider array of subsurface microbial habitats[45–47].

To date, investigations of the deep biosphere have focused on the present day distribution of subsurface life[8–10,22–24]. These studies, largely based on the microbiological analysis of cores below the sea floor and in continental aquifers, show the consistent presence of microbial communities down to several kilometres depth[14–16]. However, several recent studies have shown that there is also a geological record of the deep biosphere[18,19,48,49], to which the data from sand injectites may be added. Armed with this growing body of evidence, we can start to address fundamental questions about the antiquity of the deep biosphere, its relative importance compared with surface life, the precedence of surface or subsurface life, and also the implications for the possible distribution of life on other planetary bodies.

Methods

Sulphur isotope analysis. Pyrite samples from injectites were prepared for conventional sulphur isotopic analysis by heavy liquid and hand picking techniques. Heavy liquid separations were undertaken using suspension in bromoform. Sulphide separates were then analysed by standard techniques[50]. 5 to 10 mg were utilised for isotopic analysis. SO_2 gas was liberated by combusting the sulphides with excess Cu_2O at 1075°C, *in vacuo*. Liberated gases were analysed on a VG Isotech SIRA II mass spectrometer, and standard corrections applied to raw $\delta^{66}SO_2$ values to produce true $\delta^{34}S$. In addition to the conventional analysis, *in situ* laser combustion of sulphides was carried out on polished blocks for more detailed analyses, following the technique outlined in ref. 51. Experimental work has shown that the laser combustion results in a small but significant fractionation of the $\delta^{34}S$ values of the resulting SO_2 gas compared to the mineral $\delta^{34}S$ (e.g. ref. 51). This fractionation has been applied to the raw $\delta^{34}S$ data, and all tabulated data are thus corrected ($\delta^{34}S_{pyrite} = \delta^{34}S_{SO2}$ laser + 0.8‰; ref. 51). All SO_2 gases were analysed on a VG Isotech SIRA II mass spectrometer. Reproducibility for laser combustion is comparable to conventional analyses, being around ±0.3‰ (ref. 51). The standards employed were the international standard NBS-123 and IAEA-S-3, and SUERC standard CP-1. These gave $\delta^{34}S$ values of +17.1‰, −31.6‰ and −4.6‰ respectively, with 1σ reproducibility better than ±0.2‰ around the time of these analyses. Data (Supplementary Data 1; Fig. 4) are reported in $\delta^{34}S$ notation as per mil (‰) variations from the Vienna Canyon Diablo Troilite (V-CDT) standard.

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Author contributions

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